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## Angiotensin-converting enzyme inhibitors modulate the activation of the tissue factor pathway within aortic valves in patients with aortic stenosis: Links between blood coagulation and inflammation\*

Wpływ inhibitorów konwertazy angiotensyny na aktywację drogi krzepnięcia zależnej od czynnika tkankowego w zastawkach aortalnych u pacjentów ze stenozą aortalną: związek z zapaleniem i aktywacją układu krzepnięcia

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### Summary

**Background:** Similarities between the pathobiology of aortic stenosis (AS) and atherosclerosis have led to the concept that pharmacological strategies effective in atherosclerosis might attenuate valvular inflammation.

**Objective:** The objective of this study was to assess how angiotensin-converting enzyme inhibitors (ACEIs) might affect valvular expression of coagulation and inflammatory proteins in AS.

**Material/Methods:** We studied 111 advanced AS patients (62 males, aged 63.3±11.2 years) undergoing valve replacement. Plasma levels, valvular expression and mRNA transcripts of tissue factor (TF), TF pathway inhibitor (TFPI), prothrombin, along with C-reactive protein (CRP) and interleukin-6 (IL-6) were evaluated.

**Results:** TF-, TFPI-, CRP and prothrombin valvular expression was not related to demographics, concomitant diseases or plasma TF, free-TFPI or IL-6. ACEIs-treated patients (n=37), mainly due to hypertension (n=24, 65%), showed decreased areas for valvular TF (13.64±6.43 vs. 18.05±6.81%, p=0.03), TFPI (32.6±7.8 vs. 49.15±9.5%, p<0.001), prothrombin (23.47±1.93 vs. 26.61±1.4%, p<0.001), CRP (0.75 [0-9] vs. 1.4 [0-8]%, p=0.009), and IL-6 (3.2±0.65 vs. 6.4±1.83%, p<0.001) compared with non-ACEIs patients. Similarly, patients treated with ACEIs showed lower mRNA expression of TF (1.22±0.47 vs. 2.27±1.42, p=0.041), prothrombin (0.13±0.07 vs. 0.81±0.37, p<0.001), CRP (0.73±0.29 vs. 1.25±0.69, p=0.04), and IL-6 (7.6±5.16 vs. 13.67±7.3, p=0.046).

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<b>Conclusions:</b>	In patients with severe AS, ACEIs use is associated with lower expression of valvular proteins involved in coagulation and inflammation, thus ACEIs therapy could be important in altering atherosclerosis-like processes within stenotic aortic valves.
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INTRODUCTION

Aortic stenosis (AS) has a prevalence of 1-3% in patients older than 65 years [20]. The development and progression of AS is an active inflammatory process, sharing several common characteristics with atherosclerosis, including disruption of the basement membrane, sub-endothelial accumulation of intracellular lipids, and molecular mediators of calcification, together with infiltration of the inflammatory cells and activation of local and systemic inflammation [15, 25]. The concept of AS as an atherosclerosis-like process is supported by a number of studies showing that the development of AS is associated with cardiovascular risk factors such as smoking, hypercholesterolemia or arterial hypertension [19]. The clinical and histological similarities between the active pathobiology of AS and atherosclerosis have led to the therapeutic concept that pharmacological strategies effective in atherosclerosis, such as treatment with angiotensin-converting enzyme inhibitors (ACEIs), might reduce the progression of AS. However, available ACEIs studies yielded conflicting results, including negative findings from a large prospective and randomized trial [24]. The presence of angiotensin-converting enzyme (ACE) and angiotensin II, which cannot be found in normal valve tissue, has been demonstrated in sclerotic aortic valves [22]. This observation suggests a role for the renin-angiotensin system in the pathogenesis of aortic valve lesions. It is known that ACEIs, apart from being very effective in the current treatment of arterial hypertension therapy [3, 30], exhibit beneficial effects beyond lowering blood pressure, including anti-inflammatory [10] and antithrombotic [7] effects.

Observations made *in loco* within human aortic valves [2, 17, 18] and in an *in vivo* animal model of aortic valve disease [14] demonstrated increased expression of tissue factor (TF), a major initiator of blood coagulation *in vivo*, associated with macrophage infiltration [2, 18] of the stenotic aortic valve leaflets. It has been shown that

TF expression within aortic valves is positively associated with transvalvular pressure gradient in AS patients, which might contribute to AS progression [18]. Moreover, interleukin-6 (IL-6) [29] and C-reactive protein (CRP) [26] were found within aortic valves and the amounts of these pro-inflammatory proteins correlated with the AS severity [29].

We sought to investigate whether ACEIs influence the expression of TF, TF pathway inhibitor (TFPI) and prothrombin as well as CRP and IL-6 in patients with advanced AS without clinically overt atherosclerotic vascular disease.

**MATERIALS AND METHODS**

**Patients**

A total of 111 consecutive patients (62 men and 49 women) undergoing isolated elective aortic valve replacement for severe AS (mean transvalvular gradient  $\geq 40$  mmHg) were recruited. The exclusion criteria were: diabetes mellitus, acute infection, renal failure, Valsalva sinus aneurysm or rheumatic AS, angiographically documented epicardial artery stenosis  $>20\%$  diameter, known cancer, autoimmune disorders, endocarditis, previous cardiac surgery, a history of myocardial infarction, stroke, venous thromboembolism or bleeding [13]. Patients who required additional surgical intervention or had other heart defects were ineligible.

Information on the presence or absence of cardiovascular risk factors, including arterial hypertension, hyperlipidemia, smoking, and use of statin, ACEIs,  $\beta$ -adrenolytic drugs and acetylsalicylic acid was obtained before surgery. Smoking was defined as the use of 1 or more cigarettes per day. Arterial hypertension was diagnosed based on a history of hypertension (blood pressure  $>140/90$  mmHg) or preadmission antihypertensive treatment. Hyperlipidemia was diagnosed based on

medical records, statin therapy, or total cholesterol 5.2 mmol/l or more. Renal failure was diagnosed based on having at least 2 random fasting creatinine levels of >110 µmol/l for male and >80 µmol/l for female.

Patients were classified as receiving ACEIs if they were receiving medication at least for 6 months (ACEIs<sup>+</sup> group). The remaining patients were classified as the ACEIs<sup>-</sup> group. The Local Ethical Committee in Krakow approved the study, and the participants provided informed consent in accordance with the Declaration of Helsinki.

### Echocardiography

A transthoracic echocardiography was performed in each patient using a MargotMac 5000 ultrasound machine prior to surgery by using conventional techniques in accordance with the guidelines of European Society of Cardiology (ESC) and Polish Cardiac Society [12]. The aortic valve area (AVA) was calculated using the standard continuity equation [28]. The transvalvular gradient was measured by Doppler echocardiography using the modified Bernoulli equation [28].

### Laboratory tests

Fasting venous blood was drawn from patients 24 hours before surgery between 7 and 9 AM. Citrated blood samples (9:1 of 0.129 M citrate) were centrifuged at 2000 g at 20°C for 20 min and stored in aliquots at -80°C until analysis. Lipid profile, glucose, and creatinine were assayed by routine laboratory techniques. High-sensitivity CRP was determined using immunoturbidimetry (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Commercially available ELISA tests were used to determine in citrated plasma TF (American Diagnostica Inc., Stamford, CT, USA), and free (f)-TFPI (Diagnostica Stago, Asnieres, France) and in serum IL-6 (R&D Systems, Inc., Minneapolis, USA) according to manufacturer's instructions. All measurements were performed by technicians blinded to the origin of the samples. Intra-assay and inter-assay coefficients of variation were <8%.

### Analysis of aortic valves

Diseased aortic valves were collected during surgery for valve replacement. Valves embedded in Tissue Tec-OCT compound (Sakura, Torrance, CA, USA) for tissue cryopreservation were cryosectioned (10 µm thick) onto SuperFrost slides (Menzel-Glaser, Germany) by a Leica Jung CM 3000 cryostat. Sections were taken transversely from the mid of the leaflet and from commissural areas. Slides were stored at -20°C until immunostaining.

### Immunostaining analysis

Immunostaining was performed on adjacent sections of aortic valves as described before [17, 18]. In brief, endogenous alkaline phosphatase and peroxidase activities were quenched and unspecific background was

blocked. Primary adequate monoclonal antibodies were applied overnight at 4°C against human TF (R&D Systems, Inc., Minneapolis, USA), TFPI (American Diagnostica Inc., Stamford, CT, USA), prothrombin (Hyphen Bio Med, Neuville-Sur-Oise, France), CRP (Santa Cruz Biotechnology, Inc., CA, US) or inflammation marker – IL-6 (Abcam, Cambridge, UK). Primary antibodies were followed by the corresponding secondary antibodies conjugated with fluorochrome (Abcam, Cambridge, UK) or by the avidin-biotin complex immunoperoxidase according to manufacturer's instructions (Santa Cruz Biotechnology Inc., CA, US). Diamminobenzidine was used as the chromogen. A negative control (without primary antibody incubation) was included routinely. Sections were viewed in Zeiss, Berlin, Germany. Photomicrographs taken using a Canon A640 camera were analyzed with image analysis software Cell Analyst [11].

### Nucleic acid extraction

Human valves obtained during aortic valve replacement were immediately placed in RNAlater (Qiagen, Chatsworth, CA) and stored in liquid nitrogen. Frozen valve cups were pulverized using Mikro-Dismembrator S (Sartorius Stedim Biotech, Germany). Total RNA was isolated with the RNeasy Mini kit, according to the manufacturer's protocols (Qiagen, Chatsworth, CA) and digested with DNase (DNase I recombinant, RNase-Free, Qiagen, Chatsworth, CA). The RNA concentration was estimated by A260 measurement, and the samples were stored at -80°C.

### Quantification of TF, prothrombin, CRP and IL-6 transcripts by real-time PCR

A total of 1 µg of RNA from each individual valve cups was reverse transcribed to single strain cDNA using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems) at 25°C for 5 min, 42°C for 30 min followed by 85°C for 5 min. The cDNA was amplified with TaqMan Gene Expression Assays containing both primers and probe (TF, assay ID Hs01076032\_m1; prothrombin, Hs00354679\_m1; IL-6, Hs00985639\_m1; and CRP, Hs00265044\_m1) on an ABI PRISM® 7900HT Fast Real-Time PCR System (Applied Biosystems). The assays were from Applied Biosystems as Inventoried or Made-to-Order.

Beta-actin (Hs99999903\_m1, human ACTB Endogenous Control FAM/MGB Probe, Non-Primer Limited; Applied Biosystems) was used as a housekeeping gene. To analyze the obtained data, the comparative threshold cycle (CT) method was applied [5]. In brief, the amount of a target gen, normalized to ACTB, and relative to a calibrator (normal valve leaflet), is given by  $2^{-\Delta\Delta C_T}$ , where  $\Delta\Delta C_T = \Delta C_T$  of a sample (the  $C_T$  of the target gene of AS valves subtracted from the  $C_T$  of ACTB) -  $\Delta C_T$  of calibrator.

### Statistical analysis

Values are expressed as mean (SD) or median (interquartile range) or otherwise stated. The Kolmogor-

rov-Smirnov test was used to assess conformity with a normal distribution. Pair-wise comparisons were made using Tukey's test for continuous variables and the  $\chi^2$  test for proportions. The Mann-Whitney U test was used to compare non-normally distributed variables between two groups. Spearman's correlation coefficient was calculated to evaluate associations between variables. A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

Baseline characteristics of 111 patients are shown in Table 1.

### Immunofluorescence

Immunofluorescent staining of aortic valves showed the abundance of TF antigen at the aortic side of the leaflets, colocalizing in  $64 \pm 7.15\%$  with TFPI-positive areas

(Figure 1A). Of note, the areas positive for TFPI were 2.9-fold larger than for TF ( $49.24 \pm 11.61\%$  vs.  $18.98 \pm 9.84\%$ ). Prothrombin antigen was detected in  $25.96 \pm 12.57\%$  of the total valve area and was colocalized with both TF and TFPI (Figure 1B, C). However, areas positive for prothrombin were also visible in other, TF-free, regions. There were no age-, gender-, or smoking-related differences in the percentage of valvular areas positive for TF, TFPI, or prothrombin. We observed associations between both TF- and TFPI-positive areas and the prothrombin-positive area ( $r=0.67$ ,  $p=0.004$ ;  $r=0.71$ ,  $p=0.014$ , respectively). We also found positive correlations of TF and TFPI expression with maximal ( $r=0.62$ ,  $p<0.0001$ ;  $r=0.72$ ,  $p=0.02$ , respectively) and mean ( $r=0.53$ ,  $p=0.0002$ ;  $r=0.56$ ,  $p=0.03$ , respectively) gradients and an inverse correlation with AVA ( $r=-0.63$ ,  $p<0.0001$ ,  $r=-0.71$ ,  $p=0.004$ ), while LVEF showed an inverse association of borderline significance with TFPI ( $r=-0.50$ ,  $p=0.056$ ). Positive correlations of prothrombin valvular areas with maximal ( $r=0.51$ ,  $p=0.024$ ) and mean ( $r=0.57$ ,  $p=0.019$ ) transvalvular pressure gradients and an inverse correlation with AVA ( $r=-0.58$ ,  $p=0.011$ ) were also observed.

### Valvular mRNA expression

Analysis of relative gene expression data using real-time quantitative PCR has shown up-regulation of TF ( $2.49 \pm 1.42$ ), prothrombin ( $0.87 \pm 0.31$ ), CRP ( $1.05 \pm 1.0$ ) and IL-6 ( $8.04 \pm 6.25$ ) valvular mRNA expression within the AS valves. TF mRNA expression was positively associated with prothrombin mRNA expression ( $r=0.69$ ,  $p=0.041$ ) and plasma CRP ( $r=0.32$ ,  $p=0.027$ ), but not with plasma IL-6. However, a positive correlation between prothrombin mRNA and IL-6 mRNA expression ( $r=0.62$ ,  $p=0.045$ ) was observed. Moreover, an inverse association between expression of TF mRNA and AVA ( $r=-0.38$ ,  $p=0.012$ ) was noted.

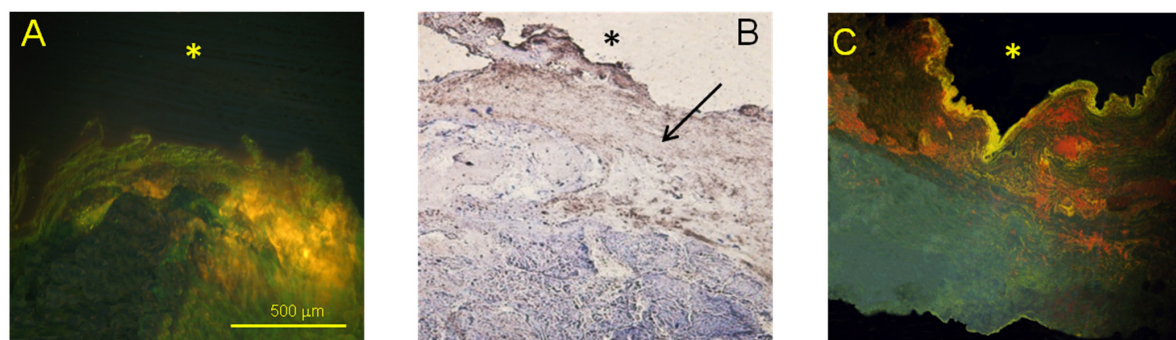
### ACEIs

Among 111 AS patients, there were 37 (33.3%) subjects treated with ACEIs, mainly due to hypertension ( $n=24$ , 65%). The following ACEIs were used: enalapril in 14 (37.8%) patients, ramipril in 15 (40.5%), and captopril in 8 (21.6%) patients. Statins were administered in 36 (32.4%) patients. No ACEIs-associated differences were observed in demographic, clinical and echocardiographic variables compared with patients not treated with ACEIs (Table 2). Compared with ACEIs<sup>-</sup> ( $n=74$ , 66.7%), ACEIs<sup>+</sup> patients were characterized by decreased percentage of valvular immunoreactive areas for TF ( $13.64 \pm 6.43$  vs.  $18.05 \pm 6.81\%$ ,  $p=0.03$ ), TFPI ( $32.6 \pm 7.8$  vs.  $49.15 \pm 9.5\%$ ,  $p<0.001$ ), prothrombin ( $23.47 \pm 1.93$  vs.  $26.61 \pm 1.4\%$ ,  $p<0.001$ ), CRP ( $0.75 [0-9]$  vs.  $1.4 [0-8]\%$ ,  $p=0.009$ ), and IL-6 ( $3.2 \pm 0.65$  vs.  $6.4 \pm 1.83$ ,  $p<0.001$ ) (Figure 2A). Valvular mRNA expression for TF ( $1.22 \pm 0.47$  vs.  $2.27 \pm 1.42$ ,  $p=0.04$ ), prothrombin ( $0.13 \pm 0.07$  vs.  $0.81 \pm 0.37$ ,  $p<0.001$ ), CRP ( $0.73 \pm 0.29$  vs.  $1.25 \pm 0.69$ ,  $p=0.04$ ), and IL-6 ( $7.6 \pm 5.2$  vs.  $13.7 \pm 7.3$ ,  $p=0.046$ ) was lower in ACEIs-treated patients compared with the non-ACEIs group (non-ACEIsure 2B).

**Table 1.** Baseline characteristics of AS patients

Variables	AS (n=111)
Male, n (%)	62 (56)
Age, years	$63.3 \pm 11.2$
Body mass index (kg/m <sup>2</sup> )	$28.7 \pm 5.1$
Risk factors	
Arterial hypertension, n (%)	24 (21.6)
Hypercholesterolemia, n (%)	37 (33.3)
Current smoking, n (%)	11 (9.9)
Obesity, n (%)	35 (31.5)
Renal failure, n (%)	23 (20.7)
Echocardiography	
Mean aortic gradient (mmHg)	$58.8 \pm 23$
Maximum aortic gradient (mmHg)	$91.9 \pm 30.6$
AVA (cm <sup>2</sup> )	$0.74 \pm 0.2$
LVEF (%)	$55.91 \pm 13.5$
Treatment	
Beta-adrenolytic drugs, n (%)	52 (46.8)
Acetylsalicylic acid, n (%)	45 (40.5)
ACE inhibitors, n (%)	37 (33.3)
Statins, n (%)	36 (32.4)
Laboratory parameters	
C-reactive protein (mg/l)	$4.7 [0.5-66.8]$
Interleukin-6 (ng/ml)	$8.5 [0.3-30.1]$
Tissue Factor (pg/ml)	$123.9 [27-192.3]$
Free Tissue Factor Pathway Inhibitor (ng/ml)	$40.6 [14.3-103]$

Data are given as mean $\pm$ SD, median (IQR) or number (percentage). LVEF, left ventricular ejection fraction; AVA, aortic valve area. Obesity was defined as body mass index  $>30$  kg/m<sup>2</sup>



**Fig. 1.** Representative micrographs of immunoreactive areas of (A), tissue factor (red) colocalized with tissue factor pathway inhibitor (green); (B), prothrombin; and (C) tissue factor (red) colocalized with prothrombin (green). Colocalized areas of each factor are stained orange. (\*) aortic side of the leaflet. Bar, 500 µm

**Table 2.** Baseline characteristics of ACEI+ vs. ACEI- patients

	ACEI+ (n=37)	ACEI- (n=74)	P value
Male, n (%)	21 (56.8)	41 (55.4)	0.99
Age, years	65.9±9.8	62.8±11.48	0.75
Body mass index (kg/m <sup>2</sup> )	30.4±4.4	27.6±4.8	0.37
Echocardiography			
Mean aortic gradient (mmHg)	56.1±17.3	58±22.4	0.71
Maximum aortic gradient (mmHg)	86.9±24.6	93.01±24.7	0.52
LVEF (%)	54.35±12.5	57.38±12.9	0.31
AVA (cm <sup>2</sup> )	0.74±0.2	0.74±0.2	0.91
Treatment			
Beta-adrenolytic drugs, n (%)	18 (48.6)	34 (45.9)	0.84
Acetylsalicylic acid, n (%)	14 (37.8)	31 (41.9)	0.84
Statins, n (%)	17 (45.9)	19 (25.7)	0.052
Laboratory parameters			
C-reactive protein (mg/l)	4.55 [0.62-51.9]	4.9 [0.5-66.8]	0.71
Interleukin-6 (ng/ml)	8.26 [1.09-17.7]	8.64 [0.31-30.07]	0.82
Tissue Factor (pg/ml)	93.08 [27-133.8]	133.08 [104.6-192.3]	0.06
Free TFPI (ng/ml)	52.3 [22.8-95.1]	41 [14.33-103]	0.01

Data are given as mean±SD, median (IQR) or number (percentage). LVEF, left ventricular ejection fraction; AVA, aortic valve area; Free TFPI, Free Tissue Factor Pathway Inhibitor

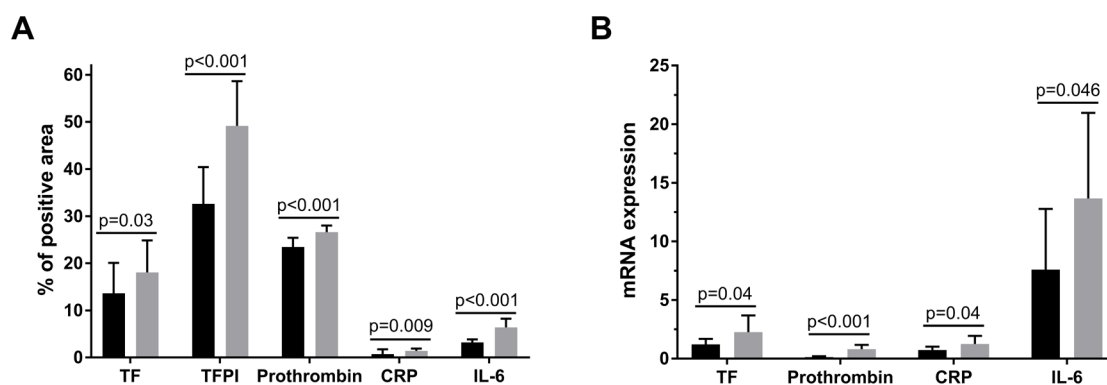
## DISCUSSION

The current study shows that long-term ACEIs use is associated with reduced expression of crucial proteins involved in both inflammation and coagulation within aortic valves in AS patients. Our major findings can be summarized as follows:

- ACEIs use is associated with a decreased percentage of valvular TF-, TFPI-, prothrombin-, CRP- and IL-6-positive areas within stenotic valves and
- mRNA expression of valvular TF and prothrombin together with CRP and IL-6 are reduced by ACEIs on a long-term treatment.

The biological mechanisms leading to AS and their similarities to atherosclerosis have typified two of the leading candidates for drug therapy in AS patients, namely statins and ACEIs. However, the available studies have failed to show the beneficial effects of statin therapy on AS progression [4, 9]. Our findings provided evidence that ACEIs could affect the valvular processes leading to valve calcification, at least in subjects with advanced AS, and they probably retard AS progression [10]. Our rationale for ACEIs-induced alterations in valvular expression of the coagulation and inflammatory proteins are based on several lines of evidence. Firstly, AS valves express Ang II and ACE [22]. Available studies have implicated the renin-angiotensin system, particularly ACE, Ang II and the Ang II type receptor, in





**Fig. 2.** Quantitative analysis of (A) immunostained prothrombin, tissue factor (TF), tissue factor pathway inhibitor (TFPI), C-reactive protein (CRP) and interleukin-6 (IL-6); (B) mRNA levels of TF, prothrombin, CRP and IL-6 within aortic valves in patients receiving (ACEI+, n=37; black columns) or non-receiving (ACEI-, n=74; gray columns) angiotensin-converting enzyme inhibitors. Bars represent means±SD or median (IQR)

AS progression, through stimulation of inflammation and macrophage activation, cholesterol accumulation, impaired fibrinolysis and increased oxidant stress [10]. Moreover, ACEIs have been shown to reduce TF expression in human blood monocytes [16]. Our study shows that TF-lowering effect of ACEIs could be potent enough to be demonstrated also in aortic valve tissue obtained from AS patients. Taubman et al. demonstrated that after angiotensin stimulation of rat aortas vascular smooth muscle cells, the expression of TF mRNA was short-lived and lasted 1 hour after stimulation [27].

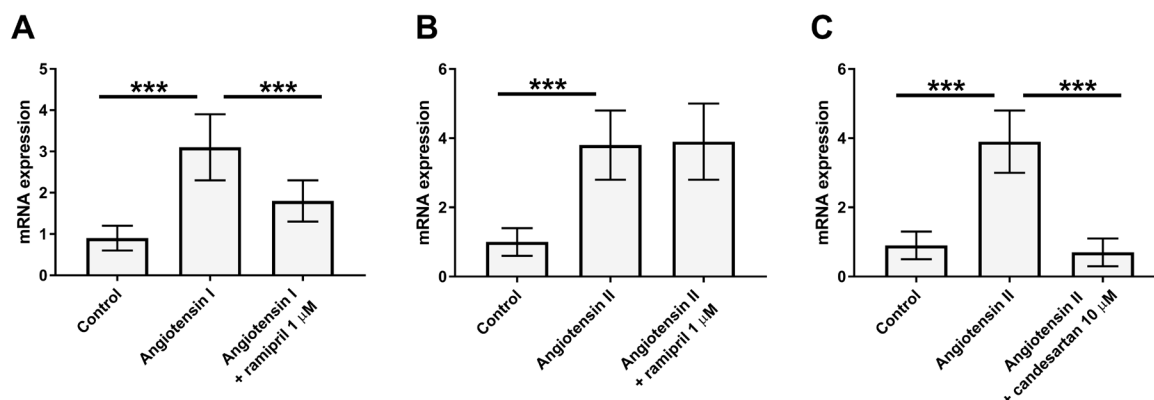
Given the data showing reduced calcium accumulation in aortic valves of AS patients receiving ACEIs [21] and those on the links between calcium and macrophage infiltration in the AS valves [2], one might suggest that ACEIs can suppress macrophage activity within the aortic valves and thus they may alter prothrombotic and proinflammatory responses within the diseased valve. It has been demonstrated in an animal model that olmesartan, an angiotensin type I receptor antagonist, reduces atherosclerotic changes in aortic valves by preserving endothelial cells integrity and inhibiting trans differentiation into myofibroblasts or into osteoblasts in valve leaflets [1]. It has also been shown that inhibition of ACE activity attenuated TF expression and microvascular remodeling in left ventricle myocardium cells of mice [31]. Finally, blood pressure lowering effect indirectly reduces the pressure overload of the left ventricle and potentially reduces the mechanical stress and strain on the aortic valve [8]. The present data might indicate that the administration of ACEIs is likely to confer potent clinical benefits in AS patients. However, the mechanisms underlying ACEIs action in AS are still unclear. Most likely, macrophages which are an integral component of stenotic valves and express Ang I and Ang II may play an important role, similarly to atherosclerosis [16]. Induction of TF gene expression in macrophages is regulated by transcriptional factors. Stimulation of

monocytes by endotoxin induces translocation of c-Rel/p65 heterodimers from the cytoplasm into the nucleus. Binding of the heterodimers to a regulatory  $\kappa$ B element within the TF promoter induces its transcriptional activation [23]. Some ACEIs, like captopril [16] have been shown to largely prevent c-Rel/p65 translocation to the promoter region of the gene encoding for TF in monocytes. Thus, the decreased expression of TF on both protein and mRNA levels in the present study may be linked with monocyte-angiotensin II biosynthesis/activity suppression.

Stenotic leaflets from patients treated with ACEIs were also characterized by decreased IL-6 expression on both protein and mRNA levels. Macrophages, which are numerous within stenotic valves and are responsible for IL-6 secretion, are released in response to angiotensin II [6]. Côté et al. showed that IL-6 induced by angiotensin II plays a key role in fibrotic response, leading to fibrocalcification of the aortic valves [6].

## STUDY LIMITATIONS

First, the presence of antigens expressed within aortic valves was determined using a semi-quantitative analysis system, which could reduce the precision of the results. However, a large number of the images analyzed per one valve likely provided reliable results. Additional analysis, such as ELISA tests performed on the valve homogenates, has not been performed. Second, the present study was focused on the valves obtained from patients with severe AS. Therefore, our observations cannot be extrapolated from subjects with mild AS or those with aortic sclerosis. Third, it is unclear whether there are any differences between various ACEIs, in terms of modulation of the expression of the proteins studied. mRNA analysis for TFPI has not been performed.



**Fig. 3.** Interleukin-6 (IL-6) mRNA expression in VICs cultures treated with angiotensin I (panel A) or II (panels B and C) and with an appropriate inhibitor

In conclusion, we have shown that ACEIs use is associated with lower expression of TF, prothrombin, CRP and IL-6 within aortic valves on both protein and mRNA levels in AS patients. Our study, indicating that long-term

ACEIs therapy could be important in altering atherosclerosis-like processes within human stenotic aortic valves, generates new hypotheses to be tested in an interventional study with a long-term follow-up.

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